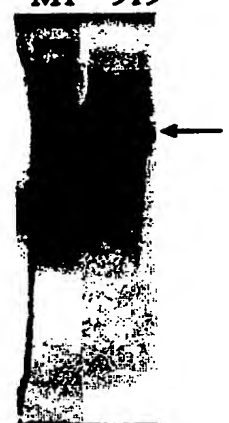




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<p>(21) International Application Number: PCT/US99/09346</p> <p>(22) International Filing Date: 30 April 1999 (30.04.99)</p> <p>(30) Priority Data:</p> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 30%;">60/083,758</td> <td style="width: 40%;">1 May 1998 (01.05.98)</td> <td style="width: 30%;">US</td> </tr> <tr> <td>60/094,869</td> <td>31 July 1998 (31.07.98)</td> <td>US</td> </tr> <tr> <td>60/098,994</td> <td>2 September 1998 (02.09.98)</td> <td>US</td> </tr> <tr> <td>60/099,062</td> <td>2 September 1998 (02.09.98)</td> <td>US</td> </tr> <tr> <td>60/103,749</td> <td>9 October 1998 (09.10.98)</td> <td>US</td> </tr> <tr> <td>60/103,794</td> <td>9 October 1998 (09.10.98)</td> <td>US</td> </tr> <tr> <td>60/103,796</td> <td>9 October 1998 (09.10.98)</td> <td>US</td> </tr> <tr> <td>60/121,528</td> <td>25 February 1999 (25.02.99)</td> <td>US</td> </tr> </table> <p>(71) Applicants (for all designated States except US): CHIRON CORPORATION [US/US]; 4560 Horton Street, Emeryville, CA 94608 (US). THE INSTITUTE FOR GENOMIC RESEARCH [US/US]; 9212 Medical Center Drive, Rockville, MD 20850 (US).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): FRASER, Claire [US/US]; Rockville, MD (US). GALEOTTI, Cesira [IT/IT]; Chiron S.p.A., Via Fiorentina, 1, I-53100 Siena (IT). GRANDI, Guido [IT/IT]; Chiron S.p.A., Via Fiorentina, 1, I-53100 Siena (IT). HICKEY, Erin [US/US]; Gaithersburg, MD (US).</p>			60/083,758	1 May 1998 (01.05.98)	US	60/094,869	31 July 1998 (31.07.98)	US	60/098,994	2 September 1998 (02.09.98)	US	60/099,062	2 September 1998 (02.09.98)	US	60/103,749	9 October 1998 (09.10.98)	US	60/103,794	9 October 1998 (09.10.98)	US	60/103,796	9 October 1998 (09.10.98)	US	60/121,528	25 February 1999 (25.02.99)	US
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<p>(US). MASIGNANI, Vega [IT/IT]; Chiron S.p.A., Via Fiorentina, 1, I-53100 Siena (IT). MORA, Marirosa [IT/IT]; Chiron S.p.A., Via Fiorentina, 1, I-53100 Siena (IT). PETERSEN, Jeremy [US/US]; Arlington, VA (US). PIZZA, Mariagratia [IT/IT]; Chiron S.p.A., Via Fiorentina, 1, I-53100 Siena (IT). RAPPUOLI, Rino [IT/IT]; Chiron S.p.A., Via Fiorentina, 1, I-53100 Siena (IT). RATTI, Giulio [IT/IT]; Chiron S.p.A., Via Fiorentina, 1, I-53100 Siena (IT). SCALATO, Enzo [IT/IT]; Chiron S.p.A., Via Fiorentina, 1, I-53100 Siena (IT). SCARSELLI, Maria [IT/IT]; Chiron S.p.A., Via Fiorentina, 1, I-53100 Siena (IT). TETTELIN, Herve [US/US]; Gaithersburg, MD (US). VENTER, J., Craig [US/US]; Rockville, MD (US).</p> <p>(74) Agent: HARBIN, Alisa, A.; Chiron Corporation, Intellectual Property - R440, P.O. Box 8097, Emeryville, CA 94662-8097 (US).</p> <p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p style="text-align: center;">Published</p> <p style="text-align: center;"><i>Without international search report and to be republished upon receipt of that report.</i></p>																										
<p>(54) Title: NEISSERIA MENINGITIDIS ANTIGENS AND COMPOSITIONS</p> <p>(57) Abstract</p> <p>The invention provides proteins from Neisseria meningitidis, including the amino acid sequences and the corresponding nucleotide sequences. The proteins are predicted to be useful antigens for vaccines and/or diagnostics.</p>																										
<p>919 (46 kDa)</p> <p>A) PURIFICATION</p> <div style="text-align: center;"> <p>M1 919</p>  </div>																										

NEISSERIA MENINGITIDIS ANTIGENS AND COMPOSITIONS

This application is a continuation-in-part of the following U.S. Provisional Patent applications, from each of which priority is claimed, and each of which is incorporated by reference in its entirety: 60/083,758 (filed May 1, 1998); 60/094,869 (filed July 31, 1998); 60/098,994 (filed September 2, 1998); 60/099,062 (filed September 2, 1998); 60/103,749 (filed October 9, 1998); 60/103,794 (filed October 9, 1998); 60/103,796 (filed October 9, 1998); and 60/121,528 (filed February 25, 1999).

This invention relates to antigens from the bacterial species: *Neisseria meningitidis* and *Neisseria gonorrhoeae*.

BACKGROUND

Neisseria meningitidis is a non-motile, gram negative diplococcus human pathogen. It colonizes the pharynx, causing meningitis and, occasionally, septicemia in the absence of meningitis. It is closely related to *N. gonorrhoea*, although one feature that clearly differentiates meningococcus from gonococcus is the presence of a polysaccharide capsule that is present in all pathogenic meningococci.

N. meningitidis causes both endemic and epidemic disease. In the United States the attack rate is 0.6-1 per 100,000 persons per year, and it can be much greater during outbreaks. (see Lieberman *et al.* (1996) Safety and Immunogenicity of a Serogroups A/C *Neisseria meningitidis* Oligosaccharide-Protein Conjugate Vaccine in Young Children. *JAMA* 275(19):1499-1503; Schuchat *et al* (1997) Bacterial Meningitis in the United States in 1995. *N Engl J Med* 337(14):970-976). In developing countries, endemic disease rates are much higher and during epidemics incidence rates can reach 500 cases per 100,000 persons per year. Mortality is extremely high, at 10-20% in the United States, and much higher in developing countries. Following the introduction of the conjugate vaccine against *Haemophilus influenzae*, *N. meningitidis* is the major cause of bacterial meningitis at all ages in the United States (Schuchat *et al* (1997) *supra*).

Based on the organism's capsular polysaccharide, 12 serogroups of *N. meningitidis* have been identified. Group A is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C are responsible for the vast majority of cases in the

United States and in most developed countries. Serogroups W135 and Y are responsible for the rest of the cases in the United States and developed countries. The meningococcal vaccine currently in use is a tetravalent polysaccharide vaccine composed of serogroups A, C, Y and W135. Although efficacious in adolescents and adults, it induces a poor immune response and short duration of protection, and cannot be used in infants [eg. Morbidity and Mortality weekly report, Vol.46, No. RR-5 (1997)]. This is because polysaccharides are T-cell independent antigens that induce a weak immune response that cannot be boosted by repeated immunization. Following the success of the vaccination against *H. influenzae*, conjugate vaccines against serogroups A and C have been developed and are at the final stage of clinical testing (Zollinger WD "New and Improved Vaccines Against Meningococcal Disease". In: *New Generation Vaccines, supra*, pp. 469-488; Lieberman *et al* (1996) *supra*; Costantino *et al* (1992) Development and phase I clinical testing of a conjugate vaccine against meningococcus A and C. *Vaccine* 10:691-698).

Meningococcus B (menB) remains a problem, however. This serotype currently is responsible for approximately 50% of total meningitis in the United States, Europe, and South America. The polysaccharide approach cannot be used because the menB capsular polysaccharide is a polymer of $\alpha(2-8)$ -linked *N*-acetyl neuraminic acid that is also present in mammalian tissue. This results in tolerance to the antigen; indeed, if an immune response were elicited, it would be anti-self, and therefore undesirable. In order to avoid induction of autoimmunity and to induce a protective immune response, the capsular polysaccharide has, for instance, been chemically modified substituting the *N*-acetyl groups with *N*-propionyl groups, leaving the specific antigenicity unaltered (Romero & Outschoorn (1994) Current status of Meningococcal group B vaccine candidates: capsular or non-capsular? *Clin Microbiol Rev* 7(4):559-575).

Alternative approaches to menB vaccines have used complex mixtures of outer membrane proteins (OMPs), containing either the OMPs alone, or OMPs enriched in porins, or deleted of the class 4 OMPs that are believed to induce antibodies that block bactericidal activity. This approach produces vaccines that are not well characterized. They are able to protect against the homologous strain, but are not effective at large where there are many antigenic variants of the outer membrane proteins. To overcome the antigenic variability, multivalent vaccines containing up to nine different porins have been constructed (eg. Poolman JT (1992) Development of a meningococcal vaccine. *Infect. Agents Dis.* 4:13-28).

Additional proteins to be used in outer membrane vaccines have been the opa and opc proteins, but none of these approaches have been able to overcome the antigenic variability (eg. Ala'Aldeen & Borriello (1996) The meningococcal transferrin-binding proteins 1 and 2 are both surface exposed and generate bactericidal antibodies capable of killing homologous and heterologous strains. *Vaccine* 14(1):49-53).

A certain amount of sequence data is available for meningococcal and gonococcal genes and proteins (e.g. EP-A-0467714, WO96/29412), but this is by no means complete. Other men B proteins may include those listed in WO 97/28273, WO 96/29412, WO 95/03413, US 5,439,808, and US 5,879,686.

The provision of further sequences could provide an opportunity to identify secreted or surface-exposed proteins that are presumed targets for the immune system and which are not antigenically variable. For instance, some of the identified proteins could be components of efficacious vaccines against meningococcus B, some could be components of vaccines against all meningococcal serotypes, and others could be components of vaccines against all pathogenic *Neisseriae* including *Neisseria meningitidis* or *Neisseria gonorrhoeae*. Those sequences specific to *N. meningitidis* or *N. gonorrhoeae* that are more highly conserved are further preferred sequences.

It is thus an object of the invention is to provide Neisserial DNA sequences which encode proteins that are antigenic or immunogenic.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the products of protein expression and purification of the predicted ORF 919 as cloned and expressed in *E. coli*.

Fig. 2 illustrates the products of protein expression and purification of the predicted ORF 279 as cloned and expressed in *E. coli*.

Fig. 3 illustrates the products of protein expression and purification of the predicted ORF 576-1 as cloned and expressed in *E. coli*.

Fig. 4 illustrates the products of protein expression and purification of the predicted ORF 519-1 as cloned and expressed in *E. coli*.

Fig. 5 illustrates the products of protein expression and purification of the predicted ORF 121-1 as cloned and expressed in *E. coli*.

Fig. 20 shows an alignment comparison of amino acid sequences for ORF 235 for several strains of *Neisseria*. Dark shading indicates regions of homology, and gray shading indicates the conservation of amino acids with similar characteristics. The Figure demonstrates a high degree of conservation among the various strains, further confirming its utility as an antigen for both vaccines and diagnostics.

Fig. 21 shows an alignment comparison of amino acid sequences for ORF 287 for several strains of *Neisseria*. Dark shading indicates regions of homology, and gray shading indicates the conservation of amino acids with similar characteristics. The Figure demonstrates a high degree of conservation among the various strains, further confirming its utility as an antigen for both vaccines and diagnostics.

Fig. 22 shows an alignment comparison of amino acid sequences for ORF 519 for several strains of *Neisseria*. Dark shading indicates regions of homology, and gray shading indicates the conservation of amino acids with similar characteristics. The Figure demonstrates a high degree of conservation among the various strains, further confirming its utility as an antigen for both vaccines and diagnostics.

Fig. 23 shows an alignment comparison of amino acid sequences for ORF 919 for several strains of *Neisseria*. Dark shading indicates regions of homology, and gray shading indicates the conservation of amino acids with similar characteristics. The Figure demonstrates a high degree of conservation among the various strains, further confirming its utility as an antigen for both vaccines and diagnostics.

THE INVENTION

The invention provides proteins comprising the *N. meningitidis* amino acid sequences and *N. gonorrhoeae* amino acid sequences disclosed in the examples.

It also provides proteins comprising sequences homologous (i.e., those having sequence identity) to the *N. meningitidis* amino acid sequences disclosed in the examples. Depending on the particular sequence, the degree of homology (sequence identity) is preferably greater than 50% (eg. 60%, 70%, 80%, 90%, 95%, 99% or more). These proteins include mutants and allelic variants of the sequences disclosed in the examples. Typically, 50% identity or more between two proteins is considered to be an indication of functional equivalence. Identity between proteins is preferably determined by the Smith-Waterman

1235

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 2605>:

m760.seq

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1   ATGGGACAGT TTATGTCAGT TTTCCGCATC AATATGACCG CCGCCACGGT
51  TTTGGCAGCA CTCTCGTCTT CGGTTTTTGC CGCACAAACG GAAGGTTTGG
101 AAACCGTCCA TATTAAGGGT CAGCGTTCTT ACAACGCGAT TGCCACCGAG
151 AAAAAACGGCG ATTACAGCTC GTTTGCCGCC ACCGTCGGTA CAAAAATCCC
201 CGCTTCTTTG CGCGAAATTC CGCAATCCGT CAGCATCATT ACCAACCAGC
251 AGGTCAAAGA CCGCAATGTT GATACGTTTG ACCAGTTGGC ACGCAAAACG
301 CCCGGCCTGC GCGTGTGAG CAACGACGAC GGACGCTCTT CGGTTTACGC
351 GCGCGGTTAC GAATACAGCG AATAACAACAT CGACGGCTG CCCGCGCAGA
401 TGCAGAGTAT CAACGGCAGC CTGCCCAACC TGTTCCGCTT CGACCGCGTG
451 GAAGTGATGC GCGGGCCGAG CGGACTGTTT GACAGCAGCG GCGAGATGGG
501 CGGCATCGTG AATCTGGTGC GCAAACGCCC GACCAAAGCG TTCCAAGGTC
551 ATGCGGCGGC AGGGTTCGGT ACGCACAAAC AATATAAAGC CGAGGCGGAC
601 GTATCGGGCA GCCTCAATTC AGACGGCAGC GTGCGCGGCC GCGTGATGGC
651 GCAGACCGTC GCGCGTCTC CGCGTCCCGC CGAGAAAAAC AACCGGCGCG
701 AAACCTTCTA CGCGGCGGCG GATTGGGACA TCAACCCCGA TACGTTTGTG
751 GCGCGGGGCT ATCTTTACCA GCAACGCCGC CTCGCGCCGT ACAACGGCCT
801 GCCTGCCGAT GCCAATAACA AATTACCGTC CCTGCCGCAA CACGTATTTG
851 TCGGCGCGGA TTGGAACAAA TTTAAATGAC ACAGCCACGA CGTGTTTCGCC
901 GATTGAAAC ATTACTTCGG CAACGGCGGC TACGGCAAAG TCGGTATGCG
951 CTATTCCGAT CGGAAAGCCG ATTCCAATTA TACGTTTTCG GGCAGCAAAC
1001 TCAACAATAC CGGACAAGCC GACGTAGCGG GTTTGGGTAC GGACATTAAA
1051 CAAAAAGCCT TTGCGGTTGA CGCAAGTTAC AGCCGTCGGT TTGCCTTGGG
1101 CAACACCGCC AACGAATTTG TGATTGGTGC AGACTACAAC CGCTTGCGCA
1151 GTACTAATGA ACAAGGGCGT TCGACTTTGT CAAAAAGCGT CGCTTTAGAT
1201 GGTTTCCGCG CTTTGCCCTTA TAACGGCATA CTTCAGAACG CCCGCGCCGG
1251 AAACAAAGGT TTCAATCACT CCGTTACCGA AGAAAACCTC GACGAAACCG
1301 GTTTGTATGC CAAGACGGTG TTCCGTCCTC TGGAAGGTTT GTCGTTGATT
1351 GCAGGCGGAC GTGTAGGACA TCACAAAATC GAGTCGGGCG ACGGCAAAAC
1401 CCTCATATAA GCTTCGAAAA CCAAATTTAC AAGCTACGCC GCGCGGGTTT
1451 ACGTATAGTA CGGCAGCAAC AGCCTGTACG CTTCCGCTC CCAACTCTAC
1501 ACACCGCAAA CCAGCATCGG CACCGACGGC AAGCTGCTCA AACCGCGCGA
1551 AGGCAACCAG TTGAAATCG GCTACAAAGG CAGCTACATG GACGACCGCC
1601 TCAATACCCG GGTTCGTTC TACCGCATGA AGGATAAAAA CGCCGCCGCA
1651 CCGCTGGACT CAAACAACAA AAAAACCCGT TACGCCGAT TGGGCAAACG
1701 CGTGATGGAA GGTGTTGAGA CCGAAATCAG CGGCGCGATG ACACCGAAAT
1751 GGCAAATCCA TGCAGGTTAC AGCTACCTGC ACAGCCAAAT CAAAACCGCC
1801 TCCAATTTCG CCGACGAAGG CATCTTCTCG CTGATGCCCA AACACAGCGC
1851 AAACCTGTGG ACGACTTACC AAGTTACGTC CGGGCTGACC ATCGGCGGGC
1901 GCGTGAACGC GATGAGCGGC ATTACTTCAT CTGCAGGGAT ACATGCAGGC
1951 GGTATGCCA CGTTCGATGC GATGGCGGCA TACCGCTTCA CGCCCAAAC
2001 GAAGCTGCAA ATCAACGCCG ACAACATCTT CAACCGCCAT TACTACGCCC
2051 GCGTCGGCAG CGAGAGCACC TTTAACATTC CCGTTTCGGA GCGCAGCCTG
2101 ACGGCAAACC TCGGTTACAG TTTTAA

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This corresponds to the amino acid sequence <SEQ ID 2606; ORF 760>:

m760.pep

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1   MGQFMSVFRI NMTAATVLAA LSSSVFAAQT EGLETVHIKQ QRSYNAIATE
51  KNGDYSSFAA TVGTKIPASL REIPQSVSII TNQQVKDRNV DTFDQLARKT
101 PGLRVLNSDD GRSSVYARGY EYSEYNIDGL PAQMQSINGT LPNLFADFVR
151 EVMRGPSGLF DSSGEMGGIV NLVRKRPTKA FQGHAAAGFG THKQYKAEAD
201 VSGSLNSDGS VRGRVMAQTV GASPRPAEKN NRRETFYAAA DWDINPDTVL
251 GAGYLYQQR LAPHYNGLPAD ANNKLPSLPQ HVFVGADWNK FKMHSHDVFA
301 DLKHYFGNGG YGKVGMRYSR RKADSNYTFA GSKLNNTGQA DVAGLGTDIK
351 QKAFAYDASY SRPFALGNTA NEFVIGADYN RLRSTNEQGR STLKSVLAD
401 GFRALPYNGI LQNRAGNKG FNHSVTEENL DETGLYAKTV FRPLEGLSLI
451 AGGRVGHKHI ESGDGKTLHK ASKTKFTSYA GAVYDIDGSN SLYASASQLY
501 TPQTSIGTDG KLLKPREGNQ FEIGYKGSYM DDRLNTRVSF YRMKDKNAAA
551 PLDSNNKKTR YAALGKRVME GVETEISGAM TPKWQIHAGY SYLHSQIKTA
601 SNSRDEGIFL LMPKHSANLW TTYQVTSGLT IGGGVNAMSG ITSSAGIHAG
651 GYATFDAMAA YRFTPKLKLQ INADNIFNRH YYARVGSEST FNIPGSERSL
701 TANLRYSF*

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CLAIMS

1. A protein comprising a fragment of an amino acid sequence from SEQ ID 2790 wherein said fragment comprises at least 7 amino acids from said sequence.
2. A protein comprising an amino acid sequence selected from the group consisting of even numbered SEQ IDs from SEQ ID number 2 through SEQ ID number 3020.
3. A protein having 50% or greater homology to a protein according to claim 1.
4. A protein comprising a fragment of an amino acid sequence selected from the group consisting of even numbered SEQ IDs from SEQ ID number 2 through SEQ ID number 3020, wherein said fragment comprises 7 or more consecutive amino acids from said sequence.
5. An antibody which binds to a protein according to any one of claims 1 to 3.
6. A nucleic acid molecule which encodes a protein according to any one of claims 1 to 3.
7. A nucleic acid molecule according to claim 5, comprising a nucleotide sequence selected from the group consisting of odd numbered SEQ IDs from SEQ ID number 1 through SEQ ID number 3019.
8. A nucleic acid molecule comprising a fragment of a nucleotide sequence selected from the group consisting of odd numbered SEQ IDs from SEQ ID number 1 through SEQ ID number 3019, wherein said fragment comprises 10 or more consecutive nucleotides from said sequence.
9. A nucleic acid molecule comprising a nucleotide sequence complementary to a nucleic acid molecule according to claim 5.
10. A nucleic acid molecule comprising a nucleotide sequence complementary to a nucleic acid molecule according to claim 6.
11. A nucleic acid molecule comprising a nucleotide sequence complementary to a nucleic acid molecule according to claim 7.
12. A composition comprising a protein, a nucleic acid molecule, or an antibody according to any preceding claim.
13. A composition according to claim 11 being a vaccine composition or a diagnostic composition.
14. A composition according to claim 11 for use as a pharmaceutical.
15. The use of a composition according to claim 11 in the manufacture of a medicament for the treatment or prevention of infection due to Neisserial bacteria.

16. A composition comprising a protein of claim 1 wherein said composition is immunogenic.

17. A composition comprising a protein of claim 2 wherein said composition is immunogenic.

18. A composition comprising a protein of claim 3 wherein said composition is immunogenic.